[function], effective to change the differentiation phenotype [manipulate the differentiation] of the contacted cell.

E1 cont

7 ~~106~~ (once amended). The method according to claim ~~90~~ 1 wherein the molecule is encoded by a gene which is a member of the ["Notch-group" of genes] Notch signaling pathway.

E2

8 ~~109~~ (once amended). A method for changing differentiation phenotype of a cell from what would otherwise occur [the manipulation of cell differentiation] comprising contacting a cell with an amount of a toporythmic protein effective to change the differentiation phenotype [manipulate the differentiation] of the contacted cell.

E3

REMARKS

Claims 90, 98, 101, 103-106 and 109-112 are presently pending in the above-captioned application, and claims 90, 98, 103, 106, 109 and 110 are presently under consideration.

Claims 90, 106 and 109 have been amended to more particularly point out and distinctly claim the subject matter of the present invention. Specifically, claim 90 has been amended to recite a method for changing differentiation phenotype of a cell from what would otherwise occur by contacting a cell with an amount of a molecule which promotes Notch signal transduction, effective to change the differentiation phenotype of the contacted cell. Claim 109 has been amended to recite a method for changing differentiation phenotype of a cell from what would otherwise occur by contacting a cell with an amount of a toporythmic protein effective to change the differentiation phenotype of the contacted cell. Support for the amended recitation of claims 90 and 109 is found in the specification as filed at page 1, line 22 to page 2, line 3; page 4, lines 19-21 and 26-31; page 5, lines 9-12, page 11, lines 22-25; and page 15, lines 14-30.

It is clear that the specification need not provide written description support in exactly the same words as are used in the claims. It is enough that the description conveys to one skilled in the art that the applicant had possession of the invention. For example, see *In re Wilder*, 736 F.2d 1516, 1520, 222 U.S.P.Q. 369, 372 (Fed. Cir. 1984):

> It is not necessary that the claimed subject matter be described identically, but the disclosure originally filed must convey to those skilled in the art that applicant has invented the subject matter later claimed.

See also *Application of Lukach*, 442 F.2d 967, 969, 169 U.S.P.Q. 795, 796 (C.C.P.A. 1971): "[T]he invention claimed does not have to be described in *ipsis verbis* in order to satisfy the description requirement of § 112." The specification on page 1, line 22 to page 2, line 3; page 4, lines 19-21 and 26-31; page 5, lines 9-12, page 11, lines 22-25; and page 15, lines 14-30 clearly conveys to one skilled in the art that the Applicant had possession of the claimed methods of changing the differentiation phenotype of a cell by promoting Notch signal transduction or contacting with a toporythmic protein.

Claim 106 has been amended to recite that the molecule is encoded by a gene which is a member of the Notch signaling pathway. Support for the amendment to claim 106 is found in the specification as filed at page 5, lines 25-31; page 12, line 27 to page 13, line 2; page 16, lines 29-30; and page 55, lines 29-30.

No new matter has been added by the amendments to the claims.

INTERVIEW SUMMARY RECORD

An interview in connection with the above-identified patent application was held at the U.S. Patent and Trademark Office on August 19, 1999 attended by Primary Examiner Dr. Yvonne Eyler, Supervisory Examiner Paula Hutzell, Dr. Spyridon Artavanis-Tsakonas, Dr. Lynne Zydowsky, Dr. Adriane M. Antler, and Mr. William Thomann. Applicant and Applicant's representatives thank Examiner Eyler and Supervisory Examiner Hutzell for their courtesy during the Interview.

All of the pending claims were discussed at the interview as were all of the Examiner's outstanding rejections. Examiner Eyler discussed with Dr. Antler the Section 112, second paragraph, rejections as well as possible amendments to the claims to overcome these rejections. Examiner Eyler suggested amending the claims to replace "manipulation of cell differentiation" with "changing the differentiation phenotype of a cell from what would otherwise occur". Regarding the term "to promote Notch function" in the claims, Examiner Eyler suggested replacing that term with "to promote Notch signal transduction". With regard to the term "Notch-group of genes", Examiner Eyler suggested instead amending the claim to recite that the gene is a member of the Notch signaling pathway.

With regard to the Examiner's allegation that the specification does not enable a molecule which promotes Notch function, Examiner Eyler indicated that this rejection would be obviated if the claims were amended in accordance with her suggestions.

Dr. Antler discussed how the claimed methods are fully enabled by the specification, and how various post-filing date publications evidenced that the specification enabled changing cell differentiation phenotype by promoting Notch signal transduction using materials either available at the filing date or using materials that had a known counterpart at the time of filing. Dr. Antler explained how in each of the post-filing date references the activation of Notch signaling, *e.g.*, by expression of an dominant active form of Notch or ligand-dependent Notch activation, resulted in inhibition of differentiation and/or misspecification of cell fate from what would otherwise occur. Drs. Artavanis-Tsakonas and Antler clarified for the Examiner that Jagged (described in Lindsell et al., 1995, Cell 80:909-917) is actually a Serrate homolog. In response to the Examiner's questions regarding any differences in manipulation of cell fate versus manipulation of cell differentiation, upon discussion with the Examiner, the point was made that a change in the differentiation phenotype of a cell is a manifestation of a change in cell fate and that the post-filing references discussed detected such a change in cell fate from what would otherwise occur by detecting a change in differentiation phenotype, *e.g.*, as evidenced by changes in cell morphology, protein expression, etc. In response to Examiner Eyler's question regarding use of the claimed method with terminally differentiated cells, Drs. Antler and Artavanis-Tsakonas also discussed evidence that Notch is expressed in terminally differentiated cells (Ahmad et al., 1995, Mech. Dev. 53:73-85) and that promotion of Notch signal transduction in terminally differentiated cells results in a change in cell differentiation phenotype of the terminally differentiated cells (Qi et al., 1999, Science 283:91-94). The Examiner asked that Qi et al. be made of record. Further details of Applicants' remarks are presented hereinbelow.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 90, 98, 103, 106, 109 and 110 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner alleges that the recitation of "the manipulation of cell differentiation" is vague and indefinite. The Examiner also alleges that the terms "Notch function" and "Notch-group" are vague and indefinite.

Applicant respectfully disagrees with the Examiner's allegations. However, as was discussed at the Interview, and in order to advance the prosecution of this application,

Applicant has amended the claims as follows such that the allegedly vague and indefinite terms are no longer recited: Claims 90 and 109 have been amended to recite that the method is to changing the differentiation phenotype of a cell from what would otherwise occur. Claims 90 and 109 have also been amended in accordance with the discussion at the interview to recite that it is Notch signal transduction that is promoted. Claim 106 has also been amended in accordance with the Examiner's suggestion to recite that the gene is a member of the Notch signaling pathway.

In view of the foregoing amendments to the claims, Applicant respectfully submits that all Section 112, second paragraph, rejections have been obviated and should be withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 90, 98, 103, 106, 109 and 110 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention for reasons of record.

Applicant respectfully disagrees with the rejection and points out that contrary to the Examiner's assertions, it is clear that Notch plays a role in cell fate and differentiation and that the differentiation phenotype of a cell can be changed from what would otherwise occur by promoting Notch signal transduction.

As evidence of the foregoing, the Examiner's attention is invited to the following publications, discussed in detail below:[1]

(1) Lindsell et al., 1995, Cell 80:909-917, ("Lindsell") (reference CQ of record);

(2) U.S. Patent No. 5,780,300 to Artavanis-Tsakonas et al., ("the '300 patent") (reference CS of record);

(3) Fortini et al., 1993, Nature 365:555-557, ("Fortini") (reference CW of record);

(4) Sakano et al., 1997, International Patent Publication WO 97/19172, ("Sakano") (reference CR of record);

---

[1] References (1)-(7) were previously made of record and were discussed in the Reply under 37 C.F.R. § 1.111 with Amendment filed on December 23, 1998. Reference (8) is attached hereto as Exhibit A, and is made of record herewith pursuant to the Examiner's request at the Interview for this application on August 19, 1999.

(5) Kopan et al., 1994, Development 120:2385-2396, ("Kopan") (reference CO of record);

(6) Nye et al., 1994, Development 120:2421-2430, ("Nye") (reference CP of record);

(7) Coffman et al., 1993, Cell 73:659-671, ("Coffman") (reference BM of record); and

(8) Qi et al., 1999, Science, 283:91-94, ("Qi") (attached as Exhibit A hereto and reference CZ made of record in the Third Supplemental Information Disclosure Statement submitted concurrently herewith).

The Examiner's attention is invited to Lindsell which clearly shows that upon promotion of Notch signal transduction by ligand binding, differentiation into muscle cells (myogenesis) is prevented, as seen by cell morphology and protein expression. In this instance, the Notch ligand used to activate Notch signal transduction is Jagged, which is the rat homolog of *Drosophila* Serrate. Moreover, on page 915, left column, bottom paragraph, Lindsell states that when the extracellular portion of Notch is deleted, muscle differentiation is perturbed (fragments of Notch lacking the extracellular domain or comprising the intracellular domain are disclosed in the specification, *inter alia*, at page 16, line 22 and at page 5, line 14).

The Examiner's attention is also invited to the '300 patent which also indicates that promotion of Notch signal transduction results in a change in cell differentiation phenotype. The '300 Patent, at column 32, lines 20-26, states:

> While the wild-type Notch gene is expressed in and required for normal development of all or most eye disc cells (Cagan and Ready, 1989, Genes Dev. 3:1099-1112; Fehon et al., 1991, J. Cell Biol. 113:657-669), a constitutively activated Notch receptor lacking the extracellular and transmembrane domains expressed under the sevenless gene control blocks cell-fate commitment, preventing ELAV expression in neural precursors and causing cell-fate misspecifications among the sevenless-expressing cells (Fortini et al., 1993, Nature 365:555-557).

(Fragments of Notch lacking the extracellular domain or comprising the intracellular domain are disclosed in the specification, *inter alia*, at page 16, line 22 and at page 5, line 14.)

The evidence presented in Fortini also demonstrates that expression of a constitutively active form of Notch which lacks the extracellular and transmembrane domains inhibits neural differentiation in the *Drosophila* eye (page 555, right column). Moreover, Fortini on page 556, right column, first full paragraph, proposes that "Notch activation may

keep cells in an undetermined state and that activated Notch should cause differentiation delays in *Drosophila*" and that their analysis provides direct evidence to support this proposition (fragments of Notch lacking the extracellular domain or comprising the intracellular domain are disclosed in the specification, *inter alia*, at page 16, line 22 and at page 5, line 14).

The Examiner's attention is also invited to Sakano which discloses experiments in which chimeric human Delta and Serrate proteins were used to change the differentiation phenotype of progenitor cells. The Examiner's attention is invited to Working Examples 10, 11 and 12 on pages 46-53 of the English translation of Sakano submitted with the Japanese language counterpart in the Second Supplemental Information Disclosure Statement. Example 10 shows both chimeric human Delta and Serrate proteins suppressed differentiation of blood progenitor cells by suppressing colony formation. The employed expression vector, HDEXIg, encodes a chimeric Delta protein that is a fusion of full length human Delta and the Fc portion below the hinge portion of human IgG (the engineering of the chimeric protein is described on pages 35-36 of the English translation). The chimeric Serrate protein, encoded by HSEXIg, was a similar fusion (its construction is described on page 39 of the English translation). Example 11 shows that both chimeric Delta and Serrate proteins used above also suppressed differentiation in long term liquid cultures of colony forming undifferentiated blood cells. Example 12 shows that the same chimeric human Delta and Serrate proteins also maintained LTC-IC cells in culture, which cells are believed to be the most undifferentiated blood cell group. Thus, the accumulated data show that Notch signaling plays a fundamental role in the differentiation of uncommitted cells, and that the promotion of Notch signal transduction results in a change of the differentiation phenotype of the cell (proteins that interact with Notch, *e.g.*, proteins that comprise the portions of Delta and Serrate that mediate binding to Notch, are disclosed in the instant specification as agonists of Notch function at page 14, line 18 and page 5, line 13).

The Examiner's attention is also invited to Kopan which shows that a constitutively active form of Notch, Notch IC, represses muscle cell differentiation (myogenesis) in mouse cells and in frog embryos.

The Examiner's attention is further invited to Nye, which shows that NotchIC not only represses myogenesis but neurogenesis as well. Nye also shows that over expression of full-length Notch suppressed neurogenesis (full length Notch protein is disclosed in the

specification in Figure 13 and is disclosed as an agonist on page 5, line 13 of the present specification). This suppression of neurogenesis is manifested as inhibition of assumption of a neural differentiation phenotype.

The Examiner's attention is further invited to Coffman which shows that a fragment of Notch lacking the extracellular domain suppressed notochord differentiation in midline cells of the mesoderm that normally give rise to the notochord; neighboring cells in which the Notch fragment was not expressed differentiated normally (page 661, left column). The differentiation of both ectodermally and mesodermally derived cells appeared to be affected by the expression of the Notch fragment (page 661, right column). Coffman concludes in the paragraph bridging pages 665-666 that the fragment of Notch lacking the extracellular domain inhibits the differentiation of ectodermal cells and that the Notch fragment is acting to inhibit such cells from committing to certain cell fates resulting in the misspecification of cell fate (and thus differentiation phenotype).

> These results demonstrate that *XotchΔE* [the deletion construct of *Xenopus* Notch lacking extracellular sequences] inhibits the differentiation of ectodermal cells into tissue derivatives that normally form soon after the completion of gastrulation and support the idea that *XotchΔE* is acting to inhibit cells from committing to these fates. *XotchΔE*, by extending competence and inhibiting differentiation, could divert a population of cells fated to become epidermis or neural crest into the muscle or neural lineages.[2]

The Examiner's attention is also invited to Qi which shows that mouse cortical neurons, which are terminally differentiated cells, when contacted with a Notch agonist that is a soluble fragment of *Drosophila* Delta (a fragment consisting of the extracellular domain of Delta) exhibit changes in differentiation phenotype, *i.e.*, morphological changes, as well as neurite retraction, that are the same effects seen upon ligand-dependent Notch activation in these cells as reported by Sestan et al. (Ref. 12 of Qi) (page 93, right column) (the fragment of Delta consisting of the extracellular domain is described in the specification on page 12, line 15, and is disclosed as an agonist on page 14, lines 23-24 of the present specification). The Qi reference thus shows that a molecule that promotes Notch signal transduction (which molecule is disclosed in the specification) is effective to change the differentiation phenotype of a terminally differentiated cell (even when the molecule is *Drosophila*-derived and the

---

[2] Coffman, paragraph bridging pages 665-666.

cells are murine). Further, with regard to terminally differentiated cells and Notch, the Examiner's attention is further invited to Ahmad et al., 1995, Mech. Dev. 53:73-85 (Ref. CN of record) which discloses that terminally differentiated adult retina neural cells express Notch, consistent with a role for Notch in terminally differentiated cells.

Applicant respectfully submits that the foregoing demonstrates, consistent with the teaching in the present specification, that the activation of the Notch signaling pathway (*i.e.*, promotion of Notch signal transduction), *e.g.*, by ligand binding to Notch or expression of an activated form of Notch or otherwise contacting the cell with a toporythmic protein, results in a change in the differentiation phenotype of the cell from what would otherwise occur.

With regard to the Examiner's statement that each of the seven post-filing date references rely on materials, methods and disclosures that were not available at the time of filing, Applicant respectfully disagrees. Firstly, Applicant points out that post-filing date references can be used to address the accuracy of a statement made in the specification, *i.e.*, that promoting Notch signal transduction results in a change in cell differentiation phenotype from what would normally occur. Application of Marzocchi, 439 F.2d 220, 169 U.S.P.Q. 367 (CCPA, 1971), fn. 4. Further, Applicant points out that the methods used in the post-filing references were available at the time of filing and that certain of these methods were also specifically disclosed in the specification, *e.g.*, cell aggregation assays. Further, the materials used in the post-filing date references were also known at the time of filing, or a counterpart was known from another species. Thus, using materials and methods known at the time of filing, or materials whose counterparts were available at the time of filing, the authors of the post-filing date references each showed that promotion of Notch signal transduction results in a change in the differentiation phenotype of the cell from what would otherwise occur, as Applicant has taught in the specification.

With regard to the Examiner's statement that the specification provides insufficient objective evidence that Delta binding was known or reasonably expected to promote Notch function, Applicant invites the Examiner's attention to the Qi reference discussed above. Qi on page 93, right column, discloses that a terminally differentiated neuronal cell contacted with a fragment of Delta consisting of the extracellular domain

resulted in a change in differentiation phenotype, the same phenotypic change reported by Sestan et al. (Ref. 12 of Qi) from ligand-dependent Notch activation in the same cells.[3]

With regard to the Examiner's statement that no other manipulations of cell differentiation aside from inhibition or prevention are taught, Applicant respectfully disagrees and points out that, at a minimum, the '300 Patent, Fortini, Kopan and Coffman each teach not only that cell differentiation is inhibited but that misspecification of cell fate results when Notch signal transduction is promoted. In other words, when the cell in which Notch is activated does not differentiate as it would normally, the cell takes on a different cell fate and thus differentiation phenotype.

With regard to the Examiner's statement that the enablement requirement has not been met for any molecule which promotes Notch function, Applicant points out that at the Interview, Examiner Eyler stated that once the claims were amended in accordance with her suggestions, the rejection based on lack of enablement for a molecule which promotes Notch function would be rendered moot.[4] Applicant points out that the claims have been amended in accordance with the Examiner's suggestions and respectfully submit that this rejection for lack of enablement has been rendered moot since the amended claims fully meet the requirements of Section 112, first paragraph.

With regard to the Examiner's statement that Applicant's argument regarding the similarities between *Drosophila* and human Notch is not persuasive, Applicant points out the following:

(1) evidence leading to the conclusion that human Notch plays a role in determining cell fate (differentiation), specifically, in view of the known role of the *Drosophila* Notch protein in determining cell fate[5], and (i) significant sequence identity

---

[3] The inconsistency between the results of Sun et al., 1997, Development 124:3439-3448 (Ref. 13 of Qi), referred to by the Examiner on page 7 of the Office Action, which show a Delta fragment acting as an antagonist of Notch, can be explained by the fact that the two fragments are not identical, as explained by Qi on page 93, right column.

[4] Applicant also points out the enabling description in the specification for such molecules. See, *e.g.*, page 5, lines 9-17; page 12, lines 8-18; page 14, lines 14-24; page 16, lines 14-24; page 48, lines 8-12; page 51, lines 16-22; page 52, line 29 to page 53, line 4; and page 53, lines 20-29.

[5] *See, e.g.*, Artavanis-Tsakonas, 1988, Trends in Genetic 4:95-100 (reference AW of (continued...)

indicative of functional conservation between *Drosophila* and human Notch proteins, not only over the entire protein length of approximately 2700 amino acids (see Second Rule 132 Declaration, ¶ 5.5.2; Fourth Rule 132 Declaration, ¶ 8.3), but also between the various functional domains (Second Rule 132 Declaration, ¶ 5.5.3) and in the identical relative arrangement of functional domains throughout the molecules (see Fourth Rule 132 Declaration, ¶¶ 8.1-8.2); (ii) the experimentally proven functional equivalence of the ligand binding domains between human and *Drosophila* Notch proteins (Second Rule 132 Declaration, ¶¶ 5.1-5.1.2 and Exhibit E); (iii) mutations in both human and *Drosophila* Notch proteins result in abnormal cell fate (differentiation) (Second Rule 132 Declaration, ¶ 5.2.1); and (iv) the developmental expression of Notch in vertebrates is similar to that in *Drosophila*, in that both are expressed in developing tissues in which cell-cell interactions are critical (Second Rule 132 Declaration, ¶ 5.3.1);

(2) evidence demonstrating that increased levels of human Notch in human tissue are associated with the presence of a malignancy (cancer) in such tissue; and

(3) references (1)-(8) discussed hereinabove.

Applicant respectfully reminds the Examiner that the evidence presented by the Applicant "need not be conclusive, but merely convincing to one skilled in the art." Manual of Patent Examining Procedure, Seventh Ed., July 1998, Section 2164.05 (emphasis in the original).

In view of the foregoing, Applicant respectfully submits that the claimed invention is fully enabled and that it would not require undue experimentation to change the differentiation phenotype of a cell by contacting the cell with a molecule that promotes Notch signal transduction or by contacting the cell with an amount of a toporythmic protein effective to change the differentiation phenotype of the cell.

CONCLUSION

Applicant respectfully requests that the amendments and remarks of the present response be entered and made of record in the file of the above-captioned application. Claims 90, 98, 101, 103-106 and 109-112 fully meet all statutory requirements for

---

[5](...continued)
record); specification, p. 1, line 22 through p. 2, line 4; Second Rule 132 Declaration, ¶ 5.2.1.

patentability. Withdrawal of the Examiner's rejections, allowance and action for issuance are respectfully requested.

Applicant respectfully requests that the Examiner call Adriane M. Antler at (212) 790-2247 if any questions or issues remain.

Respectfully submitted,

Date September 17, 1999

Adriane M. Antler 32,605
Adriane M. Antler (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090